Primary Evaluator	George F. Kramer, Ph.D., Senior Chemist Risk Assessment Branch 1 (RAB1) Health Effects Division (HED) (7509P)	Date: 29-JUN-2017
Approved by	Christine L. Olinger, Acting Branch Chief RAB1/HED (7509P)	Date: 29-JUN-2017

Note: This DER was originally prepared under contract by Versar, Inc. (6850 Versar Center, Springfield, VA 22151; submitted 6/12/12). The DER has been reviewed by HED and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT:

48677301 Dohn, D.; Chu, J. (2011) Pyrethrin I Confined Rotational Crop Study with One Radiolabel. Project Number: 2047W, 2047W/1. Unpublished study prepared by PTRL West, Inc. and Excel Research Services, Inc. 189 p.

EXECUTIVE SUMMARY:

The Consumer Specialty Products Association, Inc. (CSPA) on behalf of The Pyrethrins Joint Venture (PJV) has submitted a confined rotational crop study with [1-cyclopropane-^{14}C]pyrethrin I (specific activity 1.47 MBq/mg). The radiolabeled test substance was combined with formulation blank, containing piperonyl butoxide, and water, and applied to bare sandy loam soil at a rate equivalent to 0.70 lb ai/A (780-789 g ai/ha). Rotational crops (lettuce, radish, and wheat) were planted at plantback intervals (PBIs) of 30 and 120 days after soil treatment. Crops were harvested at maturity or the appropriate growth stage (for immature lettuce and wheat forage and straw). The in-life phase of the study was conducted at Excel Research Farm (north of Madera, CA), and the analytical phase of the study was conducted by PTRL West, Inc. (Hercules, CA).

Total radioactive residues (TRR) determined by combustion/liquid-scintillation counting (LSC) accumulated at ≥0.01 ppm in all rotated crop matrices planted 30 and 120 days following application of [^{14}C]pyrethrin I directly to bare soil at 0.70 lb ai/A. TRR, determined by summing extractable and nonextractable radioactivity, ranged from 0.010 ppm (radish root) to 0.112 ppm (wheat forage) in the 30-day matrices and from 0.010 ppm (wheat hay) to 0.035 ppm (wheat forage) in the 120-day matrices. TRR were higher in all 30-day matrices than in 120-day matrices, with the exception of radish tops and root. The highest TRR were found in wheat forage and straw. TRR at the 30- and 120-day PBIs, respectively, were: 0.052 and 0.014 ppm in immature lettuce, 0.020 and 0.017 ppm in mature lettuce, 0.020 ppm (both PBIs) in radish tops, 0.010 and 0.016 ppm in radish root, 0.112 and 0.035 ppm in wheat forage, 0.050 and 0.010 (combustion/LSC only) ppm in wheat hay, 0.108 and 0.028 ppm in wheat straw, and 0.049 and 0.018 ppm in wheat grain. With the exception of 120-day wheat hay, all rotated crop matrices were subjected to further extraction and/or analysis procedures.

Extraction with acetonitrile (ACN)/water released 46.7-57.1% TRR in immature lettuce, 60.0-

64.7% TRR in mature lettuce, 70.0-75.0% TRR in radish tops, 60.0-68.8% TRR in radish root, 68.6-70.3% TRR in wheat forage, 66.0% TRR in wheat hay, 46.4-59.3% TRR in wheat straw, and 27.8-38.8% TRR in wheat grain. Hexane extraction released negligible amounts of radioactivity (≤0.003 ppm) for samples of 30-day immature lettuce, radish root, and wheat forage; therefore, this process was discontinued for the other matrices. The nonextractable residues of 30-day immature lettuce and wheat forage and straw were also subjected to: Soxhlet extraction with methanol, which released an additional 2.7-19.2% TRR; mild acid and/or base hydrolysis at room temperature, which released 0.9-3.8% TRR; and acid hydrolysis with 6 N HCl at elevated temperatures, which released 5.6-15.4% TRR. Nonextractable residues following extraction and hydrolysis procedures accounted for <0.031 ppm in all rotational crop matrices. These procedures adequately extracted the majority radioactive residues from rotational crop matrices. Extraction results were normalized; accountabilities were 98.1-101%.

Samples were extracted within 4-36 days of harvest. Samples of 30-day lettuce (immature and mature), radish tops, and wheat forage and hay were initially analyzed by high-performance liquid chromatography (HPLC) Method 1 within 15-43 days of harvest and were reanalyzed to improve resolution using HPLC Method 2 within 49-93 days of harvest; the methods differed only in the mobile phase gradient program. All other samples were analyzed by HPLC Method 2 within 9-25 days of harvest. Because samples were stored for <6 months prior to analysis, no storage stability data are required to support the study. In addition, the petitioner compared the metabolic profile of 30-day wheat forage following extraction 42 days after harvest with the metabolic profile following partial purification for isolation of an unknown metabolite, M17, conducted 312 days after harvest. The results were very similar, demonstrating the stability of the metabolic profile in wheat forage for up to 312 days in freezer storage.

Residues were characterized and quantitated in ACN/water extracts with TRR >0.01 ppm, including: 30-day immature and mature lettuce, radish tops, and wheat forage, hay, straw, and grain; and 120-day mature lettuce, radish tops and root, and wheat forage and straw. Extracts were analyzed primarily by HPLC; Pyrethrin I degradates were characterized by retention times and named as “Mxx,” where “xx” is the retention time rounded to the nearest minute.

Pyrethrin I was not identified in any rotational crop matrix at any PBI. The residues in all samples consisted of multiple components present at very low concentrations (≤0.01 ppm) with the exception of two unknown components, M17 and M20, which were present in 30-day wheat forage at 17.9% TRR (0.020 ppm) and 8.9% TRR (0.010 ppm), respectively. M17 was present in all other matrices analyzed, except 120-day wheat straw, at 1.9-14.3% TRR (0.001-0.006 ppm), and M20 was present at ≤0.005 ppm in all other matrices except 30-day mature lettuce and wheat straw. Characterization analysis of M17 by normal-phase thin-layer chromatography (TLC) following preparative HPLC resolved the degradate into six principal components, each ≤8.0% TRR (≤0.009 ppm). Remaining radioactivity in rotational crop matrices was characterized as multiple minor components (up to 11 in each matrix), none present at >0.009 ppm.

Based on the submitted confined rotational crop study, pyrethrin I-derived residues in rotational crops consisted of multiple components, each present in very small concentration (≤0.01 ppm).

No pyrethrin I was detected in any crop sample.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the confined rotational crop residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP# 393417].

COMPLIANCE:

Signed and dated Good Laboratory Practices (GLP), Quality Assurance, and Data Confidentiality statements were provided. No GLP deviations were reported which would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Pyrethrins is the collective name of the insecticidal active ingredients present in pyrethrum extracts that are obtained from the dried and ground flowers of the pyrethrum plant, *Chrysanthemum cinerariaefolium*. The CAS Registry No. for the mixture is 8003-34-7. Currently, food/feed uses are only registered for products under PC code 069001, mixed esters of (+)-trans-chrysanthemic acid and (+)-pyrethroic acid. The nomenclature of the individual pyrethrins active ingredients is presented below in Table A.1. The physicochemical properties of the refined pyrethrins extract (TGAI) are listed in Table A.2.

TABLE A.1. Pyrethrins Nomenclature.

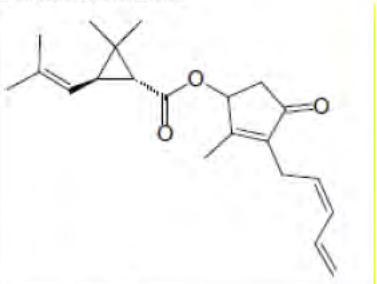
Compound	Chemical Structure
	
Common name	Pyrethrin I or Pyrethrin 1
IUPAC name	(*Z*)-(*S*)-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (1*R*,3*R*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate
CAS name	(1*S*)-2-methyl-4-oxo-3-(2*Z*)-2,4-pentadien-1-yl-2-cyclopenten-1-yl (1*R*,3*R*)-2,2-dimethyl-3-(2-methyl-1-propen-1-yl)cyclopropanecarboxylate
CAS #	121-21-1

TABLE A.1. Pyrethrins Nomenclature.	
Common name	Pyrethrin II or Pyrethrin 2
IUPAC name	(*Z*)-(*S*)-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (*E*)-(1*R*,3*R*)-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CAS name	(1*S*)-2-methyl-4-oxo-3-(2*Z*)-2,4-pentadien-1-yl-2-cyclopenten-1-yl (1*R*,3*R*)-3-[(1*E*)-3-methoxy-2-methyl-3-oxo-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate
CAS #	121-29-9
Common name	Cinerin I or Cinerin 1
IUPAC name	(*Z*)-(*S*)-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (1*R*,3*R*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate
CAS name	(1*S*)-3-(2*Z*)-2-buten-1-yl-2-methyl-4-oxo-2-cyclopenten-1-yl (1*R*,3*R*)-2,2-dimethyl-3-(2-methyl-1-propen-1-yl)cyclopropanecarboxylate
CAS #	25402-06-6
Common name	Cinerin II or Cinerin 2
IUPAC name	(*Z*)-(*S*)-3-(but-2-enyl)-2-methyl-4-oxocyclopent-2-enyl (*E*)-(1*R*,3*R*)-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CAS name	(1*S*)-3-(2*Z*)-2-buten-1-yl-2-methyl-4-oxo-2-cyclopenten-1-yl (1*R*,3*R*)-3-[(1*E*)-3-methoxy-2-methyl-3-oxo-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate
CAS #	121-20-0
Common name	Jasmolin I or Jasmolin 1
IUPAC name	(*Z*)-(*S*)-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (1*R*,3*R*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate
CAS name	(1*S*)-2-methyl-4-oxo-3-(2*Z*)-2-penten-1-yl-2-cyclopenten-1-yl (1*R*,3*R*)-2,2-dimethyl-3-(2-methyl-1-propen-1-yl)cyclopropanecarboxylate
CAS #	4466-14-2
Common name	Jasmolin II or Jasmolin 2
IUPAC name	(*Z*)-(*S*)-2-methyl-4-oxo-3-(pent-2-enyl)cyclopent-2-enyl (*E*)-(1*R*,3*R*)-3-(2-methoxycarbonylprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CAS name	(1*S*)-2-methyl-4-oxo-3-(2*Z*)-2-penten-1-yl-2-cyclopenten-1-yl (1*R*,3*R*)-3-[(1*E*)-3-methoxy-2-methyl-3-oxo-1-propen-1-yl]-2,2-dimethylcyclopropanecarboxylate
CAS #	1172-63-0

TABLE A.2. Physicochemical Properties of Refined Pyrethrins.		
Parameter	Value	Reference
Boiling point	Pyrethrin 1 = 146-148 °C at 2 x 10^{-3} Torr Pyrethrin 2 = 196-198 °C at 7 x 10^{-3} Torr Cinerin 1 = 136-138 °C at 8 x 10^{-3} Torr Cinerin 2 = 182-184 °C at 1 x 10^{-3} Torr	RD, 10/9/90, A. Smith
pH	Not applicable because the TGAI is practically insoluble in water.	
Density, bulk density, or specific gravity	0.982 g/mL at 20 °C Pyrethrin 1 = 1.5242 g/mL Pyrethrin 2 = 1.5355 g/mL	D172245, 1/5/93, C. Olinger RD, 10/9/90, A. Smith
Water solubility	<10 ppm Pyrethrin 1 = 0.00002 g/100 mL at 20 °C Pyrethrin 2 = 0.00090 g/100 mL at 20 °C	DEB No. 4525, 12/9/88, M. Flood RD, 10/9/90, A. Smith

TABLE A.2. Physicochemical Properties of Refined Pyrethrins.		
Parameter	Value	Reference
Solvent solubility	Completely soluble in nonpolar organic solvents; <0.1% in ethylene glycol Soluble in alcohol, petroleum ether, and methylene chloride	DEB No. 4525, 12/9/88, M. Flood RD, 10/9/90, A. Smith
Vapor pressure	Pyrethrin 1 = 2 x 10^{-5} mm Hg at 25 °C Pyrethrin 2 = 4 x 10^{-7} mm Hg at 25 °C	RD, 10/9/90, A. Smith
Dissociation constant (pK_a)	Not applicable because pyrethrins do not dissociate	
Octanol/water partition coefficient	Pyrethrin 1 = 5.90 K_{OW} at 25 °C Pyrethrin 2 = 4.30 K_{OW} at 25 °C	RD, 10/9/90, A. Smith
UV/visible absorption spectrum	Not available	

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

The test site was located outdoors at Excel Research Farm (north of Madera, CA). Lettuce, radish, and wheat were grown in four above-ground wooden boxes with a surface area of 1 m^2 and a soil column depth of ~18 cm. Crops were planted 30 and 120 days after a single application to bare sandy loam soil of [1-cyclopropane-^{14}C]pyrethrin I at a rate of 0.70 lb ai/A (78.0-78.9 mg per 1 m^2 plot). The petitioner indicated that a 365-day PBI was not included because of the very small concentrations of individual degradates observed in the 30- and 120-day crops and the absence of detectable amounts of pyrethrin I in any crop sample.

The crops were grown according to normal agricultural practices, and maintenance pesticides and fertilizers were applied as needed. Plots were also irrigated by hand as needed. Soil characteristics are presented in Table B.1.1, and crop information is presented in Table B.1.2.

TABLE B.1.1. Test Site Information.							
Testing Environment and location	Soil Characteristics[1]						
	Type	% Sand	% Silt	% Clay	%OM	pH	CEC (meq/100g)
Outside test plots at Excel Research Farm (north of Madera, CA)	Sandy loam	73	17	10	0.58	7.1	8.4

[1] OM = organic matter; CEC = cation-exchange capacity.

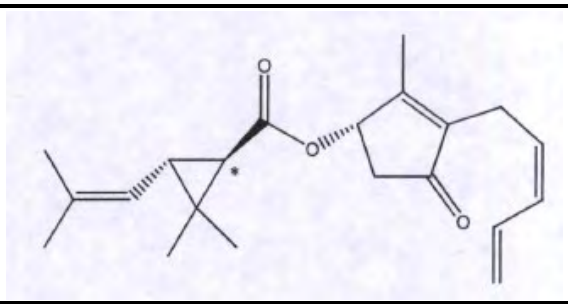
TABLE B.1.2. Crop Information.

Crop/crop group	Variety	Plant-back Intervals (days)	Growth Stage at Harvest	Harvested Matrix	Harvesting Procedure
Lettuce; Vegetable, leafy, except *Brassica*, group 4	Salad Bowl	30 and 120	Immature; 30-108 days after planting (DAP)	Leaves	Leaves cut ~2.5 cm above soil line with knife. Approximately 25% of plants collected.
			Mature; 66-179 DAP	Leaves	Leaves cut ~2.5 cm above soil line with knife. All remaining plants collected. Diseased or damaged leaves were not collected.
Radish; Vegetable, root and tuber, group 1, and Vegetable, leaves of root and tuber, group 2	Crimson Giant	30 and 120	Mature; 73-132 DAP	Root and tops	Tops were cut off with pruning shears before root was removed to prevent soil from falling on tops of plants. The remaining root was pulled/lifted from soil, gently shaken to remove loose soil, rinsed with water to remove remaining soil, and air-dried.
Wheat; Grain, cereal, group 15, and Grain, cereal, forage, fodder, and straw, group 16	Blanca Royale	30 and 120	Prior to boot stage; 30-46 DAP	Forage	Plants cut ~2.5 cm above soil line with knife. Approximately 15% of plants collected each for forage and hay samples. Hay samples were not dried in the field and were bagged in the field as green samples.
			Early flower to soft dough stage; 66-151 DAP	Hay	
			Mature; 97-210 DAP	Straw, grain, and chaff	Heads were removed from the plants by cutting with pruning shears. Straw samples were collected by cutting plants ~7.6 cm above ground. To finish processing, heads and straw were transferred to the farm building. Grain was manually separated from seed heads by rubbing seed head between hands or using threshing blocks. Seed and chaff were collected in trays, and grain was separated from chaff by using moving air to blow lighter chaff into a collection bag and allowing grain to drop into a separate collection container.

B.2. Test Materials

The test substance, [1-cyclopropane-^{14}C]pyrethrin I, was used without radio-dilution with unlabeled pyrethrin I. The test material characteristics are summarized in Table B.2.1.

TABLE B.2.1. Test Material Characteristics.

Chemical structure	
Radiolabel position	[1-cyclopropane-^{14}C]Pyrethrin I
Lot No.	197-069-013-A-20100603-PV
Purity (as total Pyrethrin I)	97.2% before application and 96.5% after application
Specific activity	13 mCi/mmol (per supplier) 8.81 x 10^7 dpm/mg (1.47 MBq/mg); (calculated as [13 mCi/mmol x 10^9 dpm/mCi]/328.2 mg/mmol)

B.3. Study Use Pattern

The radiolabeled test substance was combined with formulation blank, containing piperonyl butoxide, and water, and was applied as a broadcast spray directly to bare soil using a hand-held, pump sprayer, at a rate equivalent to 0.70 lb ai/A (780-789 g ai/ha). Rotational crops of lettuce, radish, and wheat were planted 30 and 120 days after treatment. The study use pattern is summarized in Table B.3.1.

TABLE B.3.1. Use Pattern Information.

Chemical name	[1-cyclopropane-^{14}C]Pyrethrin I
Application method	Broadcast to bare soil using hand-held sprayer
Application rate	0.70 lb ai/A (780-789 g ai/ha or 78.0-78.9 mg ai/m^2 plot)
Number of application (s)	1
Timing of application (s)	30 and 120 days prior to planting of rotational crops
Days after treatment	30 and 120

B.4. Identification/Characterization of Residues

B.4.1. Sample Handling and Preparation

Samples of immature and mature lettuce leaves were harvested by cutting above the soil line with a knife. Approximately 25% of the plants were collected for immature lettuce, and all of the remaining plants were collected for mature lettuce; diseased or damaged leaves were not collected. Samples of radish tops were cut off with pruning shears before the root was removed to prevent soil from falling on tops of plants. The remaining radish root was pulled/lifted from

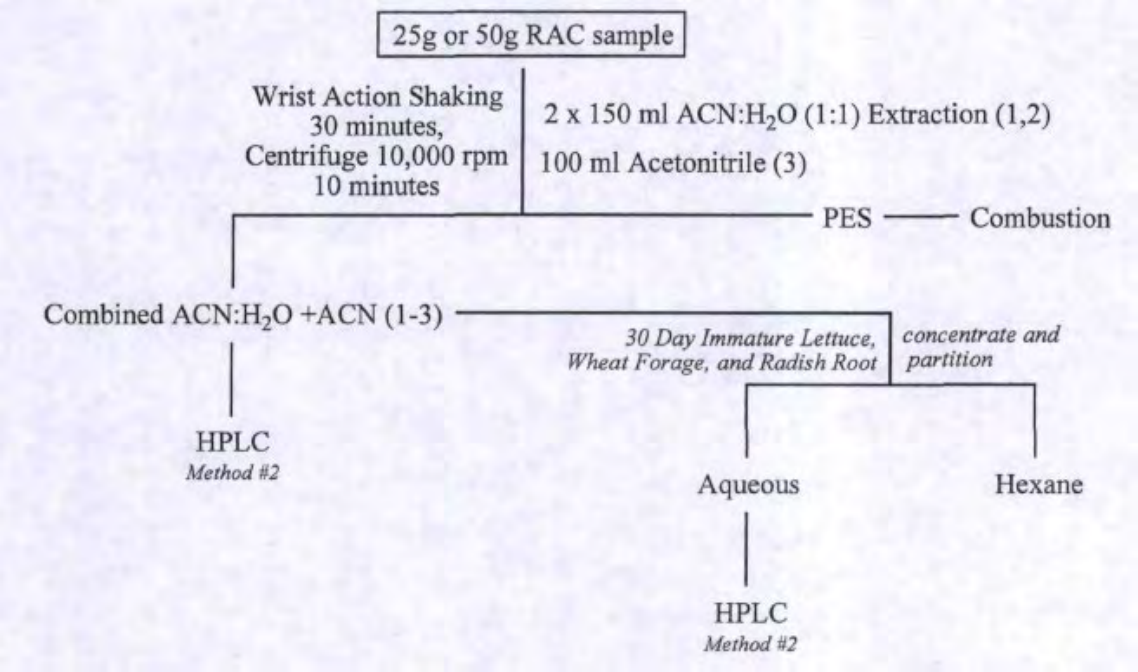
soil, gently shaken to remove loose soil, rinsed with water to remove remaining soil, and air-dried. For wheat forage and hay samples, plants were cut above the soil line with a knife. Approximately 15% of plants were collected for each forage and hay sample. Hay samples were not dried in the field and were bagged in the field as green samples. Samples of wheat grain, straw, and chaff were collected as follows: heads were removed from the plants by cutting with pruning shears. Straw samples were collected by cutting plants above ground. To finish processing, heads and straw were transferred to the farm building and grain was manually separated from seed heads by rubbing seed head between hands or using threshing blocks. Seed and chaff were collected in trays, and grain was separated from chaff by using moving air to blow lighter chaff into a collection bag and allowing grain to drop into a separate collection container. All samples were placed in frozen storage (temperature unspecified) after harvest until shipment by Excel vehicle, Federal Express, or ACDS freezer truck to the analytical laboratory, PTRL West, Inc. (Hercules, CA), where they were stored frozen (~-20 °C) until analysis. Prior to extraction and analysis, samples were homogenized in the presence of dry ice.

With the exception of wheat hay from the 30-day PBI (TRR = ≤0.010 ppm), all samples of lettuce, radish root and tops, and wheat forage, hay, grain, and straw from each plantback interval were subjected to extraction procedures. All crop samples were extracted with ACN:water (1:1, v:v; 2X) and ACN (1X), followed by centrifugation after each extraction. The three extracts were combined and concentrated for analysis. For 30-day immature lettuce, radish root, and wheat forage, the combined extracts were further subjected to liquid-liquid extraction with hexane. The petitioner indicated that this was an attempt to increase the probability of detection of pyrethrin I, which is a hydrophobic substance.

For 30-day samples with the highest residues (immature lettuce and wheat forage and straw), the nonextractable residues were subjected to Soxhlet extraction with methanol (21.5 hours). The remaining residues were dried and hydrolyzed with 1 N HCl at ambient temperature for 2 hours, followed by water at ambient temperature for 30 minutes. Solids were separated from the liquid phases by centrifugation, and the extracts were combined. The residues remaining after acid hydrolysis were subjected to the same process, using 1 N NH_4OH. The residues remaining after base hydrolysis were further subjected to acid hydrolysis with 6 N HCl at 110 °C for 7 hours. Solids were separated by centrifugation.

A representative flowchart of the extraction procedures (copied without alteration from MRID 48677301) is presented in Figure B.4.1.1.

Figure B.4.1.1. Representative Flowchart of Extraction Procedures for Rotated Crop Samples.



B.4.2. Analytical Methodology

TRR were determined by combustion/LSC in all rotational crop matrices. Extracts and hydrolysates were radioassayed by LSC, and nonextractable residues were radioassayed by combustion/LSC. In addition, TRR (in all matrices except 120-day wheat hay) were determined by summing extractable and nonextractable radioactivity. Summed TRR were used for all further calculations. The limit of quantitation (LOQ) was not reported.

Extracts of rotational crop commodities were subjected to reverse phase HPLC analysis to generate the metabolite profile. HPLC analyses were conducted using a C18 column, UV detection (226 nm), fraction collection (0.5-min intervals), and a gradient mobile phase of water and ACN each containing 0.1% formic acid. Pyrethrin I degradates were characterized by retention times and named as "Mxx" where "xx" is the retention time rounded to the nearest minute. The limit of detection for individual radioactive components was ~0.001 ppm. Samples of 30-day lettuce (immature and mature), radish tops, and wheat forage and hay were initially analyzed by HPLC Method 1; however, this method did not provide adequate resolution of the many components of the residue, and HPLC Method 2 was developed to improve resolution. The two methods differed only in the mobile phase gradient program.

Metabolite M17 of the 30-day wheat forage extract was partially purified by HPLC and analyzed by normal phase TLC. The TLC analysis was conducted using solvent systems of ethyl acetate:hexane:acetic acid (14:5:1, v:v:v and 9:9:2, v:v:v).

C. RESULTS AND DISCUSSION

The storage conditions and durations for rotational crop commodities and extracts are presented in Table C.1. The petitioner provided the dates of sample extraction and analysis. All samples were extracted within 4-36 days of harvest. Samples of 30-day lettuce (immature and mature), radish tops, and wheat forage and hay were initially analyzed by HPLC Method 1 within 15-43 days of harvest and were reanalyzed to improve resolution using HPLC Method 2 within 49-93 days of harvest. All other samples were analyzed by HPLC Method 2 within 9-25 days of harvest. Because samples were stored for <6 months prior to analysis, no storage stability data are required to support the study. In addition, the petitioner compared the metabolic profile of 30-day wheat forage following extraction 42 days after harvest with the metabolic profile following partial purification for isolation of an unknown metabolite, M17, conducted 312 days after harvest. The results were very similar, demonstrating the stability of residues in wheat forage for up to 312 days in freezer storage.

The TRR in rotational crop matrices are presented in Table C.2.1. TRR determined by combustion/LSC accumulated at ≥0.01 ppm in all rotated crop matrices planted 30 and 120 days following application of [^{14}C]pyrethrin I directly to bare soil at 0.70 lb ai/A. TRR, determined by summing extractable and nonextractable radioactivity, ranged from 0.010 ppm (radish root) to 0.112 ppm (wheat forage) in the 30-day matrices and from 0.010 ppm (wheat hay) to 0.035 ppm (wheat forage) in the 120-day matrices. TRR were higher in all 30-day matrices than in 120-day matrices, with the exception of radish tops and root. The highest TRR were found in wheat forage and straw. TRR at the 30- and 120-day PBIs, respectively, were: 0.052 and 0.014 ppm in immature lettuce, 0.020 and 0.017 ppm in mature lettuce, 0.020 ppm (both PBIs) in radish tops, 0.010 and 0.016 ppm in radish root, 0.112 and 0.035 ppm in wheat forage, 0.050 and 0.010 (combustion/LSC only) ppm in wheat hay, 0.108 and 0.028 ppm in wheat straw, and 0.049 and 0.018 ppm in wheat grain. With the exception of 120-day wheat hay, all rotated crop matrices were subjected to further analysis.

The extraction profiles and distribution of the radioactivity in rotational crop commodities are presented in Tables C.2.2.1 (lettuce), C.2.2.2 (radish), and C.2.2.3 (wheat). Extraction with ACN/water released 46.7-57.1% TRR in immature lettuce, 60.0-64.7% TRR in mature lettuce, 70.0-75.0% TRR in radish tops, 60.0-68.8% TRR in radish root, 68.6-70.3% TRR in wheat forage, 66.0% TRR in wheat hay, 46.4-59.3% TRR in wheat straw, and 27.8-38.8% TRR in wheat grain. Hexane extraction released negligible amounts of radioactivity (≤0.003 ppm) for samples of 30-day immature lettuce, radish root, and wheat forage; therefore, this process was discontinued for the other matrices. The nonextractable residues of 30-day immature lettuce and wheat forage and straw were also subjected to: Soxhlet extraction with methanol which released an additional 2.7-19.2% TRR; mild acid and/or base hydrolysis at room temperature, which released 0.9-3.8% TRR; and acid hydrolysis with 6 N HCl at elevated temperatures, which released 5.6-15.4% TRR. Nonextractable residues following extraction and hydrolysis procedures accounted for <0.031 ppm in all rotational crop matrices. These procedures adequately extracted the majority radioactive residues from rotational crop matrices. Extraction results were normalized; accountabilities were 98.1-101%.

Residues were characterized and quantitated in ACN/water extracts with TRR >0.01 ppm, including: 30-day immature and mature lettuce, radish tops, and wheat forage, hay, straw, and grain; and 120-day mature lettuce, radish tops and root, and wheat forage and straw. Extracts were analyzed primarily by HPLC.

The characterization and identification of residues in rotational crop matrices are summarized in Tables C.2.3.1 (lettuce), C.2.3.2 (radish), C.2.3.3 (wheat forage and hay), and C.2.3.4 (wheat straw and grain). Pyrethrin I was not identified in any rotational crop matrix at any PBI. The residues in all samples consisted of multiple components present at very low concentrations (≤0.01 ppm) with the exception of two unknown components, M17 and M20, which were present in 30-day wheat forage at 17.9% TRR (0.020 ppm) and 8.9% TRR (0.010 ppm), respectively. M17 was present in all other matrices analyzed, except 120-day wheat straw, at 2.8-14.3% TRR (0.001-0.006 ppm), and M20 was present at ≤0.005 ppm in all other matrices except 30-day mature lettuce and wheat straw. M17 was partially purified by HPLC and analyzed by normal-phase TLC. Most of the radioactivity associated with M17 was immobile or barely mobile in the first TLC system. The TLC plate was developed a second time in a more polar solvent system. This analysis resolved M17 into six principal components, each ≤8.0% TRR (≤0.009 ppm). Remaining radioactivity in rotational crop matrices was characterized as multiple minor components (up to 11 in each matrix), none present at >0.009 ppm.

C.1. Storage Stability

TABLE C.1. Summary of Storage Conditions.

Matrix	PBI (days)	Storage Temp. (°C)	Actual Storage Duration (days)		Interval of Demonstrated Storage Stability
			HPLC Method 1[1]	HPLC Method 2	
Lettuce, immature	30	-20	43	91	None required; samples stored <6 months.
Lettuce, mature	30		22	57	
	120			9	
Radish, tops	30		15	49	
	120			22	
	120			22	
Wheat, forage	30		42	93	
	120			23	
Wheat, hay	30		21	56	
	120			--	
Wheat, straw	30			24	
	120			24	
Wheat, grain	30			25	

[1] Shading indicates that analysis was not performed.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1. TRR in Rotational Lettuce, Radish, and Wheat.

Crop	Matrix	Residues (ppm [^{14}C]pyrethrin I)[1]			
		30-day PBI		120-day PBI	
		Combustion/LSC	Summed TRR[2]	Combustion/LSC	Summed TRR[2]
Lettuce, immature	Whole plant	0.053	0.052	0.016	0.014
Lettuce, mature	Whole plant	0.020	0.020	0.017	0.017
Radish	Tops	0.030	0.020	0.023	0.020
	Root	0.012	0.010	0.016	0.016
Wheat	Forage	0.116	0.112	0.034	0.035
	Hay	0.055	0.050	0.010	Not extracted
	Straw	0.117	0.108	0.023	0.028
	Grain	0.056	0.049	0.016	0.018

[1] Average of five replicate analyses.
[2] Normalized TRR values (sum of extractable and nonextractable radioactivity); used for all further calculations.

TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Rotational Lettuce Following Application of [^{14}C]Pyrethrin I to the Soil at 0.70 lb ai/A.[1]

Metabolite Fraction	Immature Lettuce				Mature Lettuce			
	30-day PBI		120-day PBI		30-day PBI		120-day PBI	
	TRR = 0.052 ppm		TRR = 0.014 ppm		TRR = 0.020 ppm		TRR = 0.017 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	46.7	0.024	57.1	0.008	60.0	0.012	64.7	0.011
M17	1.9	0.001			5.0	0.001	5.9	0.001
M20	1.9	0.001			--	--	5.9	0.001
Unknowns[2]	30.8	0.016			40.0	0.008	35.3	0.006
Hexane	5.2	0.003						
Nonextractable	48.1	0.025	42.9	0.006	40.0	0.008	35.3	0.006
Methanol	19.2	0.010						
1 N HCl	--	--						
1 N NH_4OH	3.8	0.002						
6 N HCl	15.4	0.008						
Nonextractable	7.7	0.004						

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Comprised of: 8 components, no single component greater than 11.5% TRR, 0.006 ppm in 30-day immature lettuce; 9 components, no single component greater than 15.0% TRR, 0.003 ppm in 30-day mature lettuce; and 7 components, no single component greater than 17.6% TRR, 0.003 ppm in 120-day mature lettuce.

TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Rotational Radish Following Application of [^{14}C]Pyrethrin I to the Soil at 0.70 lb ai/A.[1]

Metabolite Fraction	Radish Tops				Radish Root			
	30-day PBI		120-day PBI		30-day PBI		120-day PBI	
	TRR = 0.020 ppm		TRR = 0.020 ppm		TRR = 0.010 ppm		TRR = 0.016 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	75.0	0.015	70.0	0.014	60.0	0.006	68.8	0.011
M17	5.0	0.001	5.0	0.001			6.3	0.001
M20	10.0	0.002	15.0	0.003			6.3	0.001
Unknowns[2]	60.0	0.012	50.0	0.010			50.0	0.008
Hexane					--	--		
Nonextractable	25.0	0.005	30.0	0.006	40.0	0.004	31.3	0.005

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] Comprised of: 10 components, no single component greater than 15.0% TRR, 0.003 ppm in 30-day radish tops; 8 components, no single component greater than 10.0% TRR, 0.002 ppm in 120-day radish tops; and 7 components, no single component greater than 18.8% TRR, 0.003 ppm in 120-day radish root.

TABLE C.2.2.3. Distribution of the Parent and the Metabolites in Rotational Wheat Following Application of [^{14}C]Pyrethrin I to the Soil at 0.70 lb ai/A.[1]

Metabolite Fraction	Wheat Forage				Wheat Hay		Wheat Straw				Wheat Grain			
	30-day PBI		120-day PBI		30-day PBI		30-day PBI		120-day PBI		30-day PBI		120-day PBI	
	TRR = 0.112 ppm		TRR = 0.035 ppm		TRR = 0.050 ppm		TRR = 0.108 ppm		TRR = 0.028 ppm		TRR = 0.049 ppm		TRR = 0.018 ppm	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
ACN/water	70.3	0.079	68.6	0.024	66.0	0.033	59.3	0.064	46.4	0.013	38.8	0.019	27.8	0.005
M17	17.9	0.020	14.3	0.005	12.0	0.006	2.8	0.003	--	--	4.1	0.002		
Unknowns[2]	18.8	0.021	--	--	--	--	--	--	--	--	--	--		
M20	8.9	0.010	14.3	0.005	6.0	0.003	--	--	3.6	0.001	6.1	0.003		
Unknowns[3]	35.7	0.040	34.3	0.012	40.0	0.020	48.1	0.052	39.3	0.011	20.4	0.010		
Hexane	2.0	0.002												
Nonextractable	27.7	0.031	31.4	0.011	34.0	0.017	40.7	0.044	53.6	0.015	61.2	0.030	72.2	0.013
Methanol	2.7	0.003					8.3	0.009						
1 N HCl	--	--					1.9	0.002						
1 N NH_4OH	0.9	0.001					1.9	0.002						
6 N HCl	Not Analyzed						5.6	0.006						
Nonextractable	Not analyzed						24.0	0.026						

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
[2] M17 partially purified from 30-day wheat forage, comprised of 6 components, no single component greater than 8.0% TRR, 0.009 ppm.
[3] Comprised of: 7 components, no single component greater than 8.0% TRR, 0.009 ppm in 30-day wheat forage; 6 components, no single component greater than 8.6% TRR, 0.003 ppm in 120-day wheat forage; 8 components, no single component greater than 10.0% TRR, 0.005 ppm in 30-day wheat hay; 11 components, no single component greater than 8.3% TRR, 0.009 ppm in 30-day wheat straw; 9 components, no single component greater than 14.3% TRR, 0.004 ppm in 120-day wheat straw; and 6 components, no single component greater than 10.2% TRR, 0.005 ppm in 30-day wheat grain.

Table C.2.3.1. Summary of Characterization and Identification of Radioactive Residues in Rotational Lettuce Following Application of [^{14}C]Pyrethrin I to the Soil at 0.70 lb ai/A.

Compound	Immature Lettuce		Mature Lettuce			
	30-day PBI		30-day PBI		120-day PBI	
	TRR = 0.052 ppm		TRR = 0.020 ppm		TRR = 0.017 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
M17	1.9	0.001	5.0	0.001	5.9	0.001
M20	1.9	0.001	--	--	5.9	0.001
Unknowns	30.8	0.016	40.0	0.008	35.3	0.006
Hexane	5.2	0.003				
Methanol	19.2	0.010				
1 N NH_4OH	3.8	0.002				
6 N HCl	15.4	0.008				
Total identified	--	--	--	--	--	--
Total characterized	78.8	0.041	45.0	0.009	47.1	0.008
Total extractable	90.4	0.047	60.0	0.012	64.7	0.011
Unextractable (PES)[1]	7.7	0.004	40.0	0.008	35.3	0.006
Accountability[2]	98.1%		100%		100%	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

Table C.2.3.2. Summary of Characterization and Identification of Radioactive Residues in Rotational Radish Following Application of [^{14}C]Pyrethrin I to the Soil at 0.70 lb ai/A.

Compound	Radish Tops				Radish Root	
	30-day PBI		120-day PBI		120-day PBI	
	TRR = 0.020 ppm		TRR = 0.020 ppm		TRR = 0.016 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
M17	5.0	0.001	5.0	0.001	6.3	0.001
M20	10.0	0.002	15.0	0.003	6.3	0.001
Unknowns	60.0	0.012	50.0	0.010	50.0	0.008
Total identified	--	--	--	--	--	--
Total characterized	75.0	0.015	70.0	0.014	62.5	0.010
Total extractable	75.0	0.015	70.0	0.014	68.8	0.011
Unextractable (PES)[1]	25.0	0.005	30.0	0.006	31.3	0.005
Accountability[2]	100%		100%		100%	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

Table C.2.3.3. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Forage and Hay Following Application of [^{14}C]Pyrethrin I to the Soil at 0.70 lb ai/A.

Compound	Wheat Forage				Wheat Hay	
	30-day PBI		120-day PBI		30-day PBI	
	TRR = 0.112 ppm		TRR = 0.035 ppm		TRR = 0.050 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
M17	17.9	0.020	14.3	0.005	12.0	0.006
M20	8.9	0.010	14.3	0.005	6.0	0.003
Unknowns	35.7	0.040	34.3	0.012	40.0	0.020
Hexane	2.0	0.002				
Methanol	2.7	0.003				
1 N NH_4OH	0.9	0.001				
Total identified	--	--	--	--	--	--
Total characterized	67.9	0.076	62.9	0.022	58.0	0.029
Total extractable	75.9	0.085	68.6	0.024	66.0	0.033
Unextractable (PES)[1]	27.7[3]	0.031	31.4	0.011	34.0	0.017
Accountability[2]	100%[3]		100%		100%	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.
[3] The residues released from acid hydrolysis and the remaining nonextractable residues were not analyzed, therefore, the accountability is based on the sum of the residues in the ACN/water and hexane extracts and the nonextractable residues after these initial extractions.

Table C.2.3.4. Summary of Characterization and Identification of Radioactive Residues in Rotational Wheat Straw and Grain Following Application of [^{14}C]Pyrethrin I to the Soil at 0.70 lb ai/A.

Compound	Wheat Straw				Wheat Grain	
	30-day PBI		120-day PBI		30-day PBI	
	TRR = 0.108 ppm		TRR = 0.028 ppm		TRR = 0.049 ppm	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
M17	2.8	0.003	--	--	4.1	0.002
M20	--	--	3.6	0.001	6.1	0.003
Unknowns	48.1	0.052	39.3	0.011	20.4	0.010
Methanol	8.3	0.009				
1 N HCl	1.9	0.002				
1 N NH_4OH	1.9	0.002				
6 N HCl	5.6	0.006				
Total identified	--	--	--	--	--	--
Total characterized	68.5	0.074	42.9	0.012	30.6	0.015
Total extractable	76.9	0.083	46.4	0.013	38.8	0.019
Unextractable (PES)[1]	24.0	0.026	53.6	0.015	61.2	0.030
Accountability[2]	101%		100%		100%	

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

C.3. Proposed Metabolic Profile

Pyrethrin I-derived residues in rotational crops consisted of multiple components, each present in very small concentration (≤0.01 ppm). No pyrethrin I was detected in any crop sample. The proposed metabolic pathway is presented in Figure C.3.1.

FIGURE C.3.1. Proposed Metabolic Profile of Pyrethrin I in Rotational Crops.

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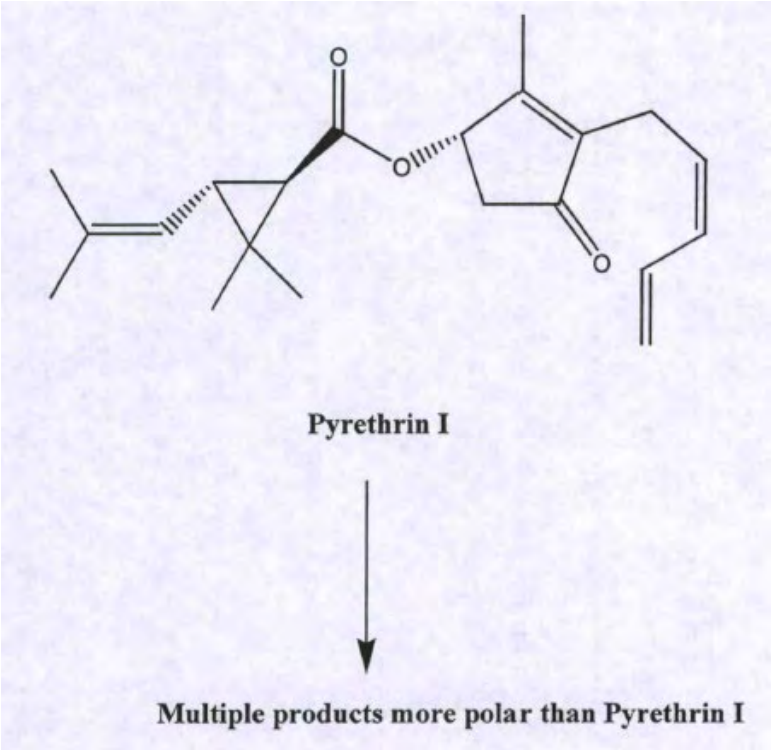

D. CONCLUSION

The submitted confined rotational crop study is acceptable. TRR accumulated at ≥0.01 ppm in all rotated crop matrices planted 30 and 120 days following application of [^{14}C]pyrethrin I directly to bare soil at 0.70 lb ai/A. TRR (determined by summing extractable and nonextractable radioactivity) at 30- and 120-day PBIs, respectively, were: 0.052 and 0.014 ppm in immature lettuce, 0.020 and 0.017 ppm in mature lettuce, 0.020 ppm (both PBIs) in radish tops, 0.010 and 0.016 ppm in radish root, 0.112 and 0.035 ppm in wheat forage, 0.050 and 0.010 (combustion/LSC only) ppm in wheat hay, 0.108 and 0.028 ppm in wheat straw, and 0.049 and 0.018 ppm in wheat grain. With the exception of 120-day wheat hay, all rotated crop matrices were subjected to further analysis.

Pyrethrin I was not identified in any rotational crop matrix at any PBI. The residues in all samples consisted of multiple components present at very low concentrations (≤0.01 ppm) with

the exception of two unknown components, referred to as M17 and M20, which were present in 30-day wheat forage at 17.9% TRR (0.020 ppm) and 8.9% TRR (0.010 ppm), respectively. M17 was present in all other matrices analyzed, except 120-day wheat straw, at 1.9-14.3% TRR (0.001-0.006 ppm), and M20 was present at ≤0.005 ppm in all other matrices except 30-day mature lettuce and wheat straw. M17 was resolved into six principal components, each ≤8.0% TRR (≤0.009 ppm). Remaining radioactivity in rotational crop matrices was characterized as multiple minor components (up to 11 in each matrix), none present at >0.009 ppm.

The study is supported by adequate storage stability data, and acceptable methods were used for the characterization and quantitation of residues.

E. REFERENCES

None.

F. DOCUMENT TRACKING

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